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Optimization of Bioethanol Production from Cassava through Fermentation Stimulant with Addition of Alpha-Amylase Enzyme

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ABSTRACT

Bioethanol, as a substitute for fuel, presents significant advantages such as environmentally friendly exhaust emissions, high octane value, and a reduction in the use of hazardous additives. The utilization of carbohydrate-rich alternatives as bioethanol feedstock is gaining prominence, particularly root vegetables like cassava. The objective of this research is to obtain bioethanol from cassava through a fermentation stimulant process involving cellulase enzyme and Saccharomyces caravisiase. In the long term, the overarching goal is to establish an alternative energy source with bioethanol production technology derived from cassava. This study comprises three treatments, incorporating the addition of alpha-amylase enzyme at 0.5, 0.1, and 0.15 mL, with a fermentation duration of 56 hours. The research findings reveal that the addition of 0.5 mL of alpha-amylase enzyme produces optimal bioethanol with the highest yield, 210 mL/kg. These results not only underscore the potential of cassava as a primary bioethanol feedstock but also make a significant contribution to the development of efficient and sustainable bioethanol production technology.

1. INTRODUCTION

The escalating concerns over the environmental impact of fossil fuels have catalyzed a global shift towards exploring alternative and sustainable energy sources (Salmahaminati et al., 2020; Purnama et al., 2019; Purnama et al., 2018; Yao et al., 2015). As the world grapples with the imperative to mitigate climate change, renewable energy options have emerged as pivotal contenders in addressing the energy crisis. Among these alternatives, bioenergy derived from biomass stands out as a promising and environmentally responsible solution (Purnama et al., 2023; Mishra et al., 2023; Hoang et al., 2022).

Fossil fuels, while historically dominant in meeting energy demands, are associated with detrimental environmental consequences, primarily the release of greenhouse gases contributing to global warming (Erickson, 2018). The urgency to transition to more sustainable energy sources has given rise to extensive research into various renewable options. Biomass, derived from organic materials such as plants and agricultural by-products, has gained traction as a renewable resource with the potential to replace or supplement traditional fossil fuels (Purnama et al., 2023).

Within the realm of biomass-based energy, bioethanol emerges as a particularly viable candidate for addressing the pressing challenges of energy security and environmental sustainability. Bioethanol, a biofuel produced through the fermentation of sugars derived from biomass, holds promise due to its lower carbon footprint compared to conventional fossil fuels

(Padil et al., 2023). As the demand for renewable energy intensifies, exploring efficient and sustainable pathways for bioethanol production becomes imperative.

Previous studies have extensively investigated the production of bioethanol from diverse biomass feedstocks, emphasizing the need for innovative approaches to enhance efficiency and cost-effectiveness (de Almeida and Colombo, 2023; Padil et al., 2023; Nazar et al., 2022; Prasasti and Herdyastuti, 2022; Tyagi et al., 2019). Cassava, with its high starch content, stands out as a noteworthy candidate for bioethanol production (Rewlay-ngoen et al., 2021; Sukandar, 2011). Furthermore, the strategic addition of alpha-amylase enzyme during fermentation has shown promise in optimizing bioethanol yields (Padil et al., 2023; Soeprijanto et al., 2022). It is important to note that the global potential of cassava is substantial. In 2020, Indonesia ranked fifth in cassava production worldwide, contributing 18.3 million tons to this versatile biomass source. This highlights the significance of exploring and refining cassava-based bioethanol technology, not only for its local implications but also for its potential impact on the global bioenergy landscape (Rizati, 2022).

In light of the foregoing, this study aims to contribute to the existing body of knowledge by exploring the optimization of bioethanol production from cassava. Through a fermentation stimulant process, we investigate the strategic addition of alpha-amylase enzyme, drawing upon insights from previous research to propel advancements in sustainable and efficient bioethanol production methodologies.

2. METHODS

This experimental study employed locally sourced cassava (Manihot utilissima) from a traditional market in Pekanbaru, processed into flour. Commercial NKL tape yeast (PT. Surya Nakula Abadi, Indonesia) served as the source of *Saccharomyces cerevisiae*, while alpha-amylase and glucoamylase enzymes were obtained from Shaanxi Fonde Biotech Co., Ltd. The research followed a Completely Randomized Design (CRD), with treatments involving the addition of alpha-amylase enzyme at volumes of 0.5, 0.1, and 0.15 mL, and a 56-hour fermentation duration. The experiment was replicated three times.

The study commenced with the sterilization of equipment and sorting of materials. Subsequently, it encompassed pretreatment, hydrolysis (liquefaction and saccharification), fermentation, and distillation. In the pretreatment phase, cassava underwent cleaning by removing its outer skin and rinsing with clean water. Cleaned cassava was diced, dried for 48 hours at 40°C, and ground to obtain cassava flour. For the liquefaction process, 100 grams of cassava flour were mixed with 250 mL of distilled water, heated to 90°C, and stirred. While maintaining a stable 80°C temperature, alpha-amylase enzyme was added, and the mixture was stirred for 1 hour. Saccharification involved cooling the liquefied solution to 60°C. Glucoamylase enzyme (0.1 mL) was added to each treatment while stirring, and saccharification occurred for 3 hours at 60°C.

In the fermentation phase, saccharified solution was transferred to Erlenmeyer flasks, and commercial *Saccharomyces cerevisiae* (4 grams per treatment) was added. Fermentation occurred for 56 hours under anaerobic conditions. For distillation, the fermented solution was centrifuged to separate it from the remaining starch slurry. Distillation was conducted at 78°C to separate ethanol from water and other by-products. Calculation of fermentation yield (R_f),

distillation yield (R_d), and ethanol concentration (B) followed equations from prior research (Lovisia, 2022).

$$R_f = \frac{Hf}{Bp} \times 100\% \quad \text{.....(1)}$$

$$R_d = \frac{Bd}{Hf} \times 100\% \quad \text{.....(2)}$$

$$B = \frac{Bd}{S} \quad \text{.....(3)}$$

Where:
R_f = Fermentation yield (%)
H_f = Volume of fermented solution (L)
B_p = Volume of starch slurry (L)
R_d = Distillation yield (%)
B_d = Volume of ethanol from distillation (L)
H_f = Volume of fermented solution (L)
B = Ethanol concentration per kilogram (L/kg)
S = Weight of cassava raw material (kg)

This comprehensive methodological approach ensures a systematic exploration of the bioethanol production process from cassava, from initial preparation to final yield calculations. Data obtained from this research were subjected to variance analysis with a significance level of 5%. This statistical analysis aimed to measure the extent of data variation within the sample and ensure the reliability of the obtained results. The results of the variance analysis were used to support the validity and reliability of the data collected during the study.

3. RESULTS AND DISCUSSIONS

The data presented in Table 1 elucidates the impact of varying alpha-amylase enzyme volumes on crucial parameters in the bioethanol production process. Analyzing this dataset reveals intricate relationships and trends that provide valuable insights into the optimization of enzyme concentrations. Commencing with the fermentation yield, we observe a gradual increase from 57.54% with 0.5 mL alpha-amylase to 61.39% with 0.15 mL, culminating in the highest yield of 69.11% with 0.1 mL alpha-amylase. This progressive improvement aligns with the expected enzymatic effect on sugar conversion during fermentation. Higher enzyme volumes tend to enhance the efficiency of *Saccharomyces cerevisiae* in converting cassava-derived sugars into ethanol.

The statistical examination of fermentation yield, derived from the three treatments involving the addition of alpha-amylase at 0.5, 0.10, and 0.15 mL, reveals a lack of significant difference in the ethanol fermentation yields obtained from cassava. This is evidenced by the statistical F value of 20.788 with a significance level of 0.002. The significance level, being less than 0.05 (at a 5% significance level), indicates a statistically significant result. The outcome of the hypothesis test suggests that the utilization of alpha-amylase enzyme in these varied volumes contributes significantly to the ethanol fermentation yield. The lack of a substantial difference among the treatments reinforces the notion that the enzyme's presence,

irrespective of the specific volume, plays a pivotal role in influencing and optimizing the fermentation process for bioethanol production from cassava.

Tabel 1. Effect of varied α-amylase enzyme volumes on fermentation yield, distillation yield, and bioethanol concentration from cassava (n = 3)

Alfa-amylase enzyme (mL)	Fermentation yield (%)	Distillation yield (%)	Ethanol concentration (mL/kg)
0.50	57.54	10.1	210
0.15	61.39	5.9	120
0.10	69.11	4.9	110

In contrast, the distillation yield exhibits a contrasting trend. While the 0.5 mL alpha-amylase treatment yields 10.1%, indicating effective ethanol recovery, the distillation yield diminishes as the enzyme volume increases. The 0.1 mL alpha-amylase treatment yields the lowest distillation yield at 4.9%. This observation suggests a potential trade-off between fermentation and distillation efficiency at higher enzyme concentrations, echoing findings in similar studies (Prasasti and Herdyastuti, 2022), where the addition of α-amylase enzyme in the fermentation process of jackfruit seeds with *Saccharomyces cerevisiae* increased the ethanol content produced.

The statistical scrutiny of distillation yield, stemming from three distinct treatments involving the addition of alpha-amylase at 0.5, 0.10, and 0.15 mL, reveals a lack of significant difference in the ethanol distillation yields obtained from cassava. This is evidenced by the statistical F value of 23.63 with a significance level of 0.001. The significance level, being less than 0.05 (at a 5% significance level), indicates a statistically significant result. The outcome of the hypothesis test indicates that the utilization of alpha-amylase enzyme in these varied volumes significantly contributes to the ethanol distillation yield. The absence of a substantial difference among the treatments reinforces the understanding that the enzyme, irrespective of its specific volume, plays a crucial role in influencing and optimizing the distillation process for bioethanol production from cassava.

The ethanol concentration per kilogram of cassava further underscores the complexity of this relationship. With 0.5 mL alpha-amylase, the concentration peaks at 210 mL/kg, indicative of a highly concentrated bioethanol product. However, as the enzyme volume increases, the concentration decreases to 120 mL/kg with 0.15 mL and further to 110 mL/kg with 0.1 mL alpha-amylase. This inverse relationship between enzyme concentration and ethanol density emphasizes the need for a delicate balance to achieve optimal ethanol concentrations in the final product (Padil et al., 2023).

The statistical examination pertaining to the ethanol concentration per kilogram of cassava, arising from three distinct treatments involving varying alpha-amylase volumes (0.5, 0.10, and 0.15 mL), does not reveal a substantial difference in the obtained ethanol concentrations from cassava. This conclusion is substantiated by a statistical F value of 20.711 with a significance level of 0.002. Importantly, the significance level, being less than 0.05 (at

a 5% significance level), underscores a statistically significant finding. The results of the hypothesis test highlight a significant contribution of alpha-amylase enzyme utilization to the ethanol concentration per kilogram of cassava. The lack of marked distinction among the treatments emphasizes that the enzyme, regardless of its specific volume, plays a crucial role in influencing and optimizing the ethanol concentration in the bioethanol product derived from cassava.

Azad et al. (2015) disclosed that *Saccharomyces cerevisiae* is essential for fermentation, but the addition of enzymes enhances starch conversion to simple sugars, accelerating the fermentation process and bioethanol production. The introduction of alpha-amylase enzyme facilitates rapid fermentation, resulting in the characteristic alcohol aroma. However, excessively high enzyme concentrations can inhibit the reaction process, reducing the amount of substrate convertible into bioethanol. The correlation between higher sugar concentrations and increased bioethanol productivity is highlighted. This phenomenon is attributed to the greater availability of substrates for *Saccharomyces cerevisiae* metabolism, resulting in a proportional increase in ethanol production (Supriyanto and Wahyudi, 2007).

Utilizing alpha-amylase enzyme volumes of 0.5, 0.1, and 0.15 mL demonstrates that employing 0.5 mL yields the highest bioethanol volume, averaging 210 mL/kg during a 56-hour fermentation. This is attributed to the increased dextrin formation during the hydrolysis process, specifically during liquefaction, which is subsequently converted into glucose for fermentation. This aligns with findings by Putri and Fachruroji (2011), linking reducing sugar levels closely to ethanol production from fermentation. While increasing substrate concentration boosts bioethanol yield, there exists a maximum substrate concentration for efficient bioethanol fermentation. Excessive sugar concentrations result in diminished bioethanol production due to substrate inhibition effects (Supriyanto and Wahyudi, 2007). The complex amylase system improves with a higher enzyme volume percentage, constrained by glucose content that can inhibit enzyme activity (Sukandar et al., 2011). Distillation yield of bioethanol in this study peaks with the use of 0.10 mL alpha-amylase, indicating that this volume does not induce inhibition.

The enzymatic process applied to solid cassava, termed hydrolysis, involves both physical and enzymatic methods. Enzymatic hydrolysis utilizes alpha-amylase and glucoamylase enzymes. Compared to chemical hydrolysis, both physical and enzymatic hydrolysis are considered environmentally friendly, aligning with Anindyawati (2009). Nevez et al. (2006) asserted that microorganisms like *Saccharomyces cerevisiae* lacking amylolytic enzymes cannot directly convert starch into ethanol. Thus, starch conversion to glucose is necessary through hydrolysis, involving liquefaction to break down starch into dextrin and saccharification to convert dextrin into simple sugars using enzymes. The addition of two enzymes, alpha-amylase for liquefaction and glucoamylase for saccharification, enhances the hydrolysis process of cassava starch into glucose. The obtained glucose is crucial for achieving high bioethanol concentrations through fermentation, as fermentation transforms glucose into alcohol (Nurdyastuti, 2008).

4. CONCLUSIONS

In conclusion, this study presents valuable insights into optimizing bioethanol production from cassava, specifically focusing on the concentration of bioethanol per kilogram of cassava. The addition of alpha-amylase enzyme in varying volumes demonstrated a nuanced impact on the final bioethanol concentrations. Notably, the treatment with 0.5 mL of alpha-amylase showcased the highest bioethanol concentration per kilogram of cassava at 210 mL/kg, suggesting the potential for fine-tuning enzyme dosages to enhance the ethanol content. These findings underline the delicate balance required in enzyme utilization to achieve optimal bioethanol concentrations, providing a pathway for future research to explore and refine this aspect of the production process. Beyond the laboratory setting, the study contributes to the broader goal of developing cassava-based bioethanol technology as a promising and sustainable alternative energy source. The emphasis on bioethanol concentration offers practical implications for the bioenergy industry, guiding efforts towards more efficient and environmentally friendly fuel production.

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